Lysostaphin

Week 1

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

To be able to actively work with Lysostaphin, we first amplified the biobrick present in the distribution kit by transformation of *E. coli* TOP 10.

1 Transformation of Lysostaphin into *E. coli* TOP 10

Responsible

Oskar Ohman and Shuangjia Xue

Protocols used

- Transformation
- Colony picking
- PCR Amplification (Taq)
- Gel electrophoresis
- Glycerol stocks

Modifications and comments to protocols

Transformation: Recovery period was adjusted to 2 hours instead of 1 hour
PCR purification: Eluted the bound DNA with 30 μl of elution buffer instead of 50 μl

Experimental Set Up

Sample: BBa K748002 Truncated Lysostaphin Coding Sequence
The experiment consisted of transformation of biobrick plasmid into *E. coli* TOP 10 cells, followed by colony picking of four colonies. To ensure that the right insert is present in the plasmid, a confirmation step was done by colony PCR using the verification primers VF2 and VR. Amplified inserts are then purified using a PCR purification kit (Qiagen) and can be digested for further manipulation.

Sample Calculation

No calculations were done.

Results and Conclusions

Transformation was successful, around 50 colonies was observed. Based on the results from the colony PCR, bands from sample Lys 1 and Lys 4 showed the best results and was later purified to obtain the values below.

Table 1: Concentrations of purified PCR product

Sample	Concentration [ng/μl]
Lyostaphin 1	89.1
Lysotaphin 4	87.9

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Discussion and Troubleshooting

The biobrick BBa K748002 consists of a 741 bp sequence that codes for truncated lysostaphin. Lysostaphin is initially produced as a pre-proenzyme (prolysostaphin) when it is released from the cell in its native host *S. simulans*. For Lysostaphin to aquire its bacteriolytic activity, it must undergo

maturation by the removal NH2-terminal portion which contains seven tandem repeats of 13-amino acid sequence (Rescei, 1987).
In this project, we have chosen to work with BBa K748002 which is a truncated version of lysostaphin coding sequence, first developed by HIT-Harbin 2012 Team and later verified by TecCEM 2015 Team.

References

Rescei, P. A., Gruss, S. D. and R. P. Novick. 1987. Cloning, sequence and expression of the lysostaphin *Staphyloccus simulans*. Proc. Natl. Acad. Sci. USA. Vol 84: 1127-1131